The Endocrine Pancreas

Introduction

The endocrine pancreas consists of three cells that make three hormones—insulin, glucagon, and somatostatin. That's the crux of it. In Biochemistry: Metabolism, we dedicated an entire topic, 18 lessons, to how insulin and glucagon oppose one another to regulate energy states. We told that story from the perspectives of the molecules—fatty acid synthesis or oxidation, gluconeogenesis or glycolysis—and the molecules they influence. We are going to revisit the subject broadly, reviewing the metabolic outcome of these hormones. But the perspective of the story changes, now focusing on the hormones, the receptors they activate, and the second messengers that lead to the metabolic changes. And whereas there we proposed the insulin-dominant world and the glucagon-dominant world, as if the two hormones were of equal import, here we define insulin as the more important of the two. As you've already seen in the Endocrine module, we've shown you the counter-regulatory hormones—cortisol, growth hormone, and epinephrine—that work with glucagon to oppose insulin's actions. We used the glucagon-vs.-insulin perspective in the Metabolism module because the first organ the pancreatic hormones see is the liver when released into the portal circulation. And because the story was told mostly from the perspective of the hepatocytes, that made sense.

Now, insulin is what matters the most. The reason for that is the disease of diabetes, which happens due to insufficient insulin or insulin resistance. Diabetes, or the complications that result from the disease, was the cause of one-fifth of all healthcare dollars spent last year. What year is that, you ask? Doesn't matter. It's been that money for a long time, and diabetes is only getting worse. Therefore, we will spend most of this lesson on insulin, insulin synthesis, the regulation of insulin release, and the insulin receptor. We will also cover the anatomy of a pancreatic islet, discuss how glucagon and other counter-regulatory hormones oppose insulin's effects and close with a discussion of excess pancreatic hormones other than insulin. In the next lesson, we'll discuss the disease diabetes, its complications, and the pathogenesis of those complications. In the third lesson, we'll cover insulin treatment—both exogenous insulin and all the drug classes we have to treat insulin disorders.

Pancreatic Islets

The pancreas consists mainly of acini attached to ducts, producing the digestive enzymes that contribute to the exocrine pancreas. Every once in a while, there is a **pancreatic islet**, a small cluster of cells that isn't an acinus. Like the acinus, the pancreatic islet is spherical in shape. But unlike the acinus, there is no duct, only blood vessels that run through the sphere. The artery penetrates the islet and emerges as fenestrated capillaries that run from the middle of the islet back out. Oxygenated blood enters the capillary at the center of the sphere. The blood then courses outward from the middle of the islet, ensuring that the cells in the core of the islet get the freshest blood supply. These cells in the center of the islet are **β cells**, and they are the ones that **make insulin**. β Cells are connected to each other by gap junctions so that they can act in concert. How they act is covered below.

The blood flows out towards the edge of the sphere. At the outer edges of the islet are two other cell types, **α cells** that secrete **glucagon** and **δ cells** that secrete **somatostatin**. Any hormones released by the pancreas into the blood are drained into the **portal circulation** and delivered in the highest concentrations to the liver. You may recall from the Metabolism lessons that the liver is the provider of energy during the fasting state and the storer of energy during the fed state. Glucagon and insulin modulate the organism, defining the destination—the insulin-dominant state or glucagon-dominant state. The liver alone is responsible for producing glucose, the source of energy, during the fasting state. Thus, it makes the most sense that the highest concentration of hormones is delivered to the liver. That arrangement also ensures clearance of the hormones. The liver metabolizes much of the hormones as first-pass metabolism, preventing inappropriate surges from entering the systemic circulation. What does get through to systemic circulation directs the rest of the body.

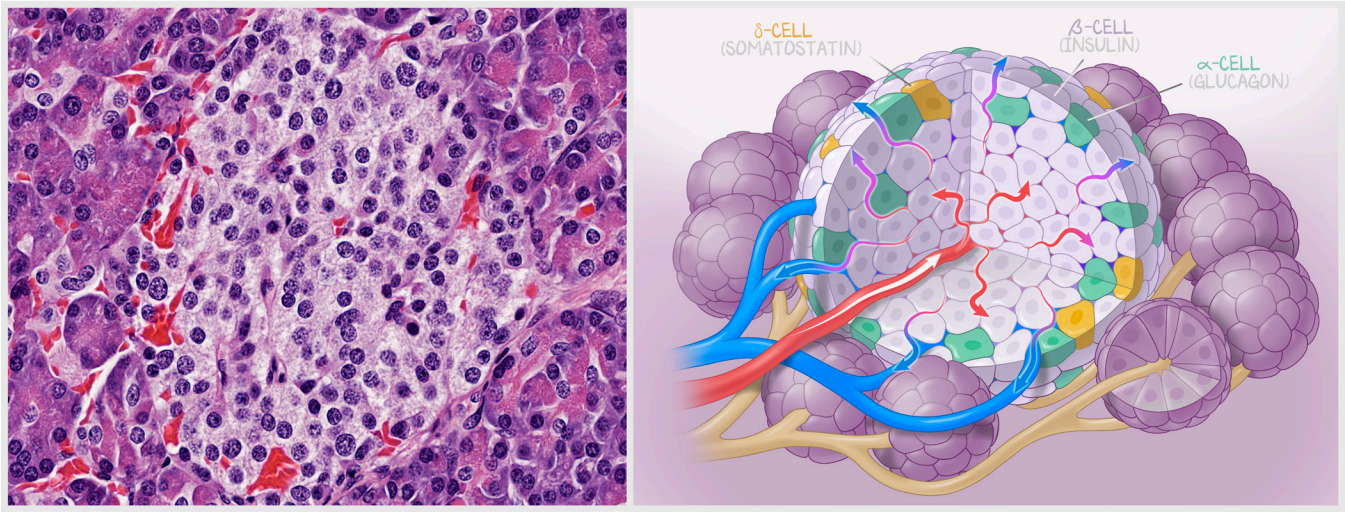

Figure 1.1: Pancreatic Islets
On the left is a high-magnification light micrograph of a pancreatic islet. The pale-staining cluster in the middle is the islet. The smaller clusters of cells with dark purple cytoplasm are the exocrine acini. Punctuating the field are red blood cells, indicating where capillaries are. On the right is an artist's rendition of an islet, demonstrating its spherical shape and its size relative to nearby exocrine acini. The arterial supply penetrates the center of the islet, and blood flows back out to the periphery. The majority of cells are β cells, and the β cells tend to be in the center of the sphere. The α cells and δ cells are sporadic and populate the outside of the islet.

Insulin Synthesis

All the peptide hormones we've encountered so far in Endocrine are synthesized in membrane-bound organelles. The only way they can get into the vesicle (membrane-bound organelle) that fuses with the plasma membrane for exocytosis is for them to be synthesized inside a membrane-bound organelle. We are reminding you of such intense biochemistry for insulin because of the utility of **C-peptide** in assessing for an insulinoma versus a patient who is injecting exogenous insulin (malingering, factitious), and because excess insulin secretion also results in excess amylin secretion in diabetes.

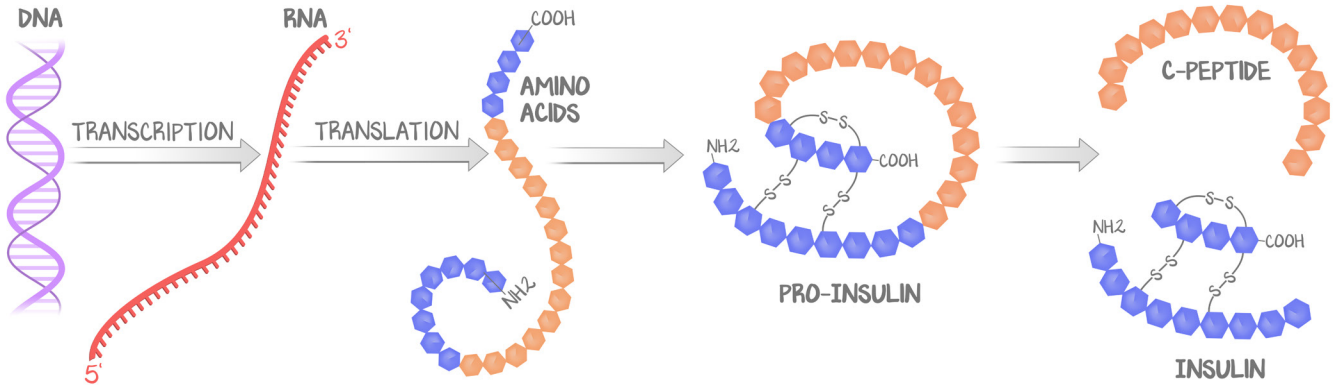

Figure 1.2: Insulin Synthesis
Preproinsulin is the amino acid sequence that includes the signal sequence that directs the ribosome to the RER to be synthesized within a membrane-bound organelle. In the RER, the amino acid sequence is completed, and the signal sequence is removed. Proinsulin is the remaining A, B, and C domains. In the Golgi apparatus, proinsulin undergoes post-translational modification. First, the folding of the molecule so that the A chain and B chain are bound by disulfide bridges, then cleavage of the C chain, resulting in both insulin and C-peptide in the budding vesicle.

The insulin gene codes for a protein with a leader sequence and three peptide segments named A, B, and C. The leader sequence ensures that the peptide's destiny is to be synthesized within a membrane-bound organelle. Ribosomes begin translation, and the leader sequence directs the ribosome to the rough endoplasmic reticulum. As the amino sequence is synthesized into the RER lumen, the leader sequence is removed, and the molecule is named **preproinsulin**. Preproinsulin is sent to the Golgi apparatus for post-translational modification. There, the ABC peptide chain has its A domain and B domain linked by **disulfide bridges**, and the molecule is now known as **proinsulin**. The final processing of insulin is to have the **C-peptide domain cleaved** from the molecule. This results in the A-B-linked-by-sulfur (**insulin**) and a C-peptide. When exocytosis occurs, both C-peptide and insulin are released.

C-peptide has no known physiological purpose. But because its half-life is much longer than that of insulin, it can be used to assess whether hypoglycemia, a symptom of excess insulin, is due to the endogenous production of excess insulin (C-peptide high) or the surreptitious injection of exogenous insulin (C-peptide low).

Insulin Release

In experimental models, **depolarization** of the β cells results in **insulin release**. Thus, it is known that depolarization is the key. This depolarization in the experimental model is induced by raising extracellular potassium, indicating that it is a potassium current that maintains hypopolarization (as is the case in all excitable cells) and that the absence of that potassium current induces depolarization.

What causes this depolarization normally? **GLUT2** glucose transporters are present in the membrane of the pancreas and liver. These allow for a small amount of glucose to enter the cell without the stimulation of insulin. GLUT2 transporters in the liver also let glucose out of the cell during gluconeogenesis. The point is that GLUT2 transporters are always active and are present on the cells that sense glucose levels in the blood. As glucose comes into the cytoplasm, it is acted upon—glycolysis, TCA, electron transport chain. The result is **increased ATP** and **increased NADH**. The rise in these high-energy molecules **closes K^+ channels**. The equilibrium potential for K^+ is –90 mV, far from the depolarization threshold. By closing the K^+ channels, the cell moves away from the equilibrium potential for K^+, thus leading to depolarization (General Physiology #6: *Excitable Cells: Passive Properties*).

It is the rise in the ATP/ADP and NADH/NAD ratios that lead to the channels' closure. That means it isn't how much glucose there is in the blood or the pancreatic β cells. That means that the presence of molecules other than glucose that can participate in [TCA-electron transport chain] can also induce insulin secretion. Other hexoses, such as fructose, and amino acids also stimulate insulin secretion. The common link is the [TCA-electron transport chain].

Depolarization of the cell leads to the opening of **L-type calcium channels** and the influx of extracellular calcium. This influx leads to **Ca^{2+}-dependent Ca^{2+} release** from the endoplasmic reticulum. The calcium levels rise such that vesicular fusion results in the release of insulin and C-peptide into the blood. This is just like you learned at the synaptic cleft. Depolarization results in calcium influx. Calcium influx induces the fusion of vesicles to the cell's plasma membrane and exocytosis of their contents into the blood.

There is additional regulation of vesicular fusion, mediated through G protein-coupled receptors. Cholecystokinin and acetylcholine activate a G protein-coupled receptor that activates the **G_q-IP_3-Ca^{2+}** receptor pathway that stimulates insulin release. **Epinephrine** and **glucagon** stimulate a G protein-coupled receptor that activates the **G_s-AC-cAMP-PKA** pathway, which leads to insulin release. The counter-regulatory hormones meant to oppose insulin's activity stimulate insulin release, acting as a fail-safe from excess counter-regulatory activity. Finally, **somatostatin** stimulates a **G_i-AC-cAMP-PKA** receptor pathway, inhibiting insulin release and antagonizing the epinephrine and glucagon signals.

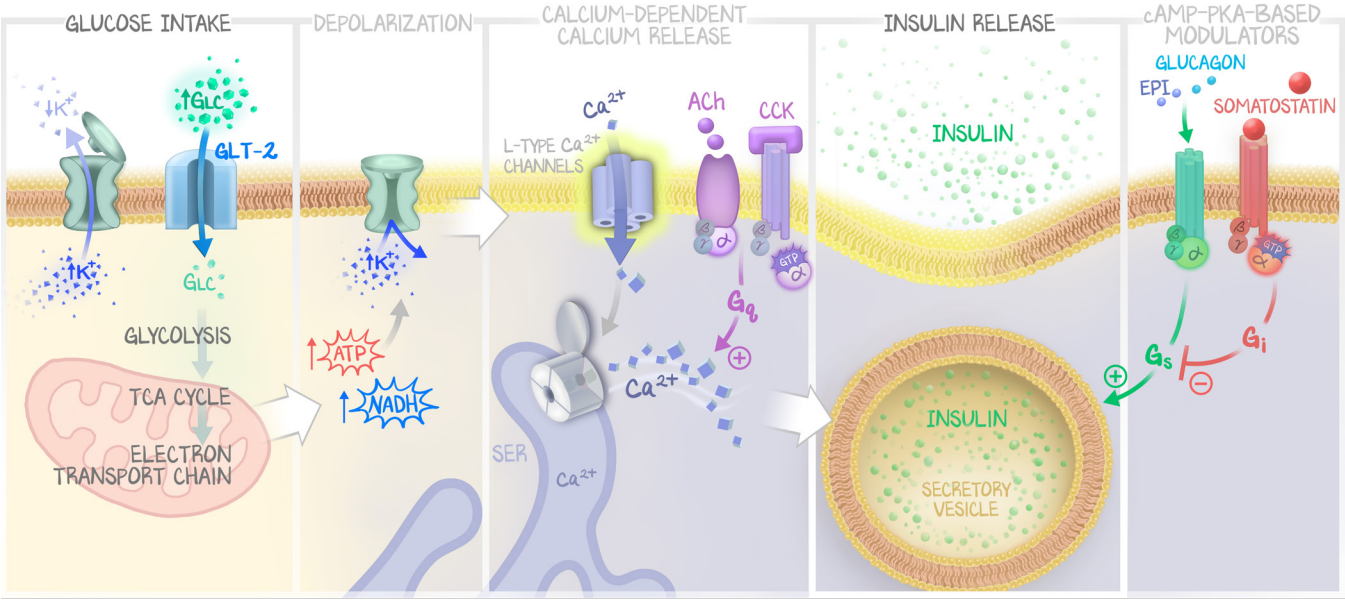

Figure 1.3: Insulin Release
The final signal that releases insulin is calcium. The glucose-driven pathway is via the closure of K^+ channels, depolarization, the opening of voltage-gated Ca^{2+} channels, and subsequent calcium-dependent calcium release from the endoplasmic reticulum. Hormonal mechanisms exist as well that are independent of depolarization and calcium-dependent calcium release. CCK and ACh use the G_q-IP_3-Ca^{2+}-PKC pathway; both IP_3's release of calcium from the endoplasmic reticulum and PKC's activity stimulate vesicle fusion. Epinephrine and glucagon (stimulatory) and somatostatin (inhibitory) act on the G_s-AC-cAMP-PKA pathway, with the PKA responsible for modulating vesicular release.

Glucagon is a potent insulin secretagogue. However, because most of the α cells are located downstream from the β cells, it is unlikely that glucagon exerts an important paracrine effect on insulin secretion. Excess glucagon may make its way back around to the β cells through the systemic circulation. The interplay between insulin and glucagon in lower organisms likely plays a role in maintaining homeostasis. In humans, insulin and glucagon come from the pancreas to influence the liver. They do not circle back around to influence the pancreas.

Insulin Release Modulation—Incretins

Ingesting glucose has more of an effect on insulin release than injecting glucose does. This discovery led to the search for additional hormones used to regulate insulin secretion beyond the effects local to the β cells. Indeed, medical science has discovered three peptides released by intestinal cells in response to feeding that enhance insulin secretion: **cholecystokinin** from I cells, **glucagon-like peptide 1** (GLP-1) from L cells, and **gastric inhibitory polypeptide** (GIP, also called glucose-dependent insulinotropic peptide) from K cells. These hormones are collectively called **incretins**. GLP-1 is degraded by an enzyme in the endothelium called DDP-4. GLP-1 analogs and DPP-4 inhibitors you will see in pharmacology. Their physiology is less well understood than the other hormones, but it seems that they prime the pancreas, increasing β cells' sensitivity to glucose levels and getting them ready for the pending glucose flood from the meal.

The Insulin Receptor on Skeletal Muscle and Adipose

The insulin receptor is a **receptor tyrosine kinase**. When insulin binds to its receptor, the receptor's cytoplasmic kinase domains activate, autophosphorylating themselves, then phosphorylate intracellular proteins. There are intermediate **insulin receptor substrates** (IRS proteins). The pathways are not

worth committing to memory, but subsequently involve second messengers you've seen before, like the RET BRAF-MAP kinase arm, the RET RAS-PI3 kinase arm, and several others. The result of insulin receptor stimulation is the **insertion of GLUT4** transporters into the cell's membrane from **cytoplasmic vesicles**, increasing glucose influx into the cell. The other effects of the insulin pathway are to **induce proliferation**, **glycogen synthesis**, fatty acid storage as triglycerides, and **protein synthesis.**

The single most important thing to know about the insulin receptor is that it **lowers blood sugar levels**. It does so through the insertion of GLUT 4 transporters into the membranes of skeletal muscle and adipocytes. In healthy subjects, a rise in blood glucose (because of a meal) stimulates insulin release. The glucose is meant to be used to store energy while energy is abundant. The blood sugar rises, the pancreas responds by releasing insulin, GLUT 4 receptors are inserted, and cells take up and trap that glucose. Insulin also manipulates cytoplasmic processes that ensure the utilization of that glucose.

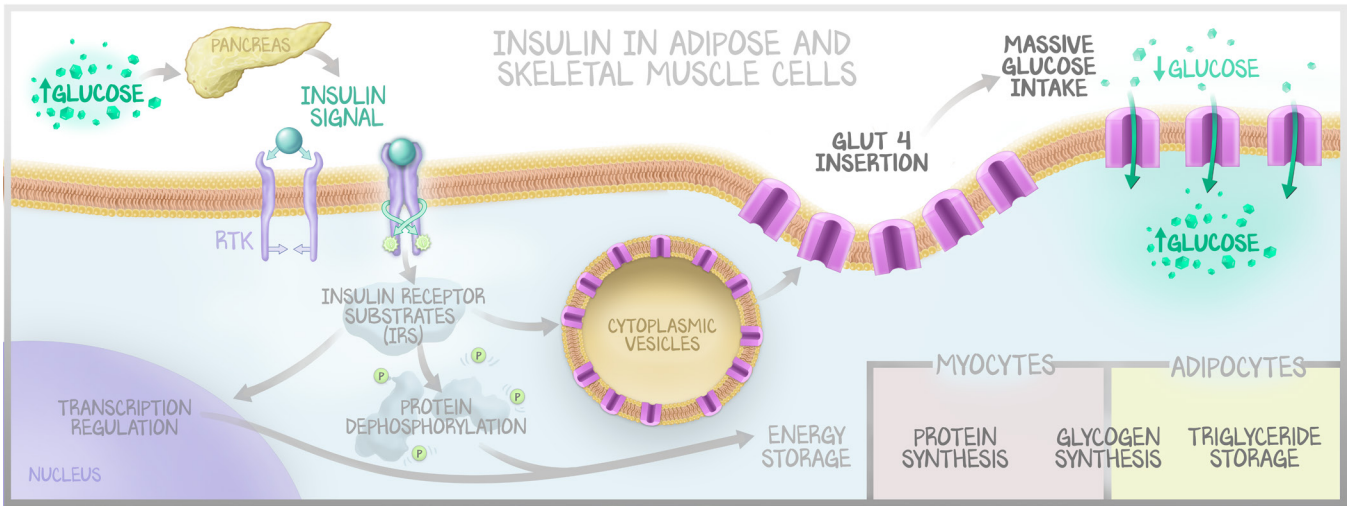

Figure 1.4: Insulin in Adipose and Skeletal Muscle
Insulin has a unique effect on the cells of metabolism. In the insulin-dominant state, the liver sends out excess energy to skeletal myocytes as amino acids and to adipocytes as fatty acids. Insulin induces changes in gene transcription and phosphorylation states to ensure the myocytes and adipocytes store those molecules for later. Building amino acids and triglycerides is insulin's anabolic effect on these tissues. But most important to understanding diabetes at the next level is the immediate effect that insulin has on GLUT4 transporters. The blood glucose level decreases in response to insulin because myocytes and adipocytes have cytoplasmic vesicles loaded with GLUT4, ready to be fused with their plasma membrane. These cells have GLUT4, and these cells are why glucose levels fall in response to insulin.

The number of insulin receptors present on a target cell membrane is determined by the balance of receptor synthesis and the receptor-mediated endocytosis and degradation of receptors. Cells chronically exposed to high insulin concentrations have fewer surface receptors than those exposed to lower concentrations. The ability of cells to increase or decrease the number of specific receptors on their surface is called **downregulation**. Insulin downregulates insulin receptors by decreasing receptor synthesis and increasing degradation. Insulin's stimulation of its receptor results in **decreased synthesis,** as well as **endocytosis and destruction** of the receptor, in addition to all the other metabolic effects. Downregulation of insulin receptors is the main pathogenic mechanism of type 2 diabetes.

The Insulin Receptor in Hepatocytes

As discussed in all of the Metabolism modules, insulin prompts the liver to switch to an insulin-dominant world. In this state, the liver no longer acts as the glucose manufacturer for the rest of the body. The liver stores energy. It stores energy within hepatocytes by **increasing glycogenesis** and

inhibiting gluconeogenesis. The liver stores energy in adipocytes by **fatty acid synthesis** and dispatches those fatty acids as VLDL to the peripheral tissue—mainly adipocytes.

Glycogen. Insulin dephosphorylates and activates **glycogen synthase** while dephosphorylating and inactivating **glycogen phosphorylase**. There is no regulation of gene transcription. Glycogen is synthesized to be used as the first source of glucose in the glucagon-dominant state.

Carbohydrates. Insulin dephosphorylates and activates **PFK-2**, making more fructose-2,6-bisphosphonate, ultimately stimulating **PFK-1** (glycolysis) and inhibiting fructose-1,6-bisphosphatase (gluconeogenesis). Insulin also stimulates pyruvate kinase and pyruvate dehydrogenase. Insulin **induces glucokinase** transcription and **inhibits PEPCK** transcription.

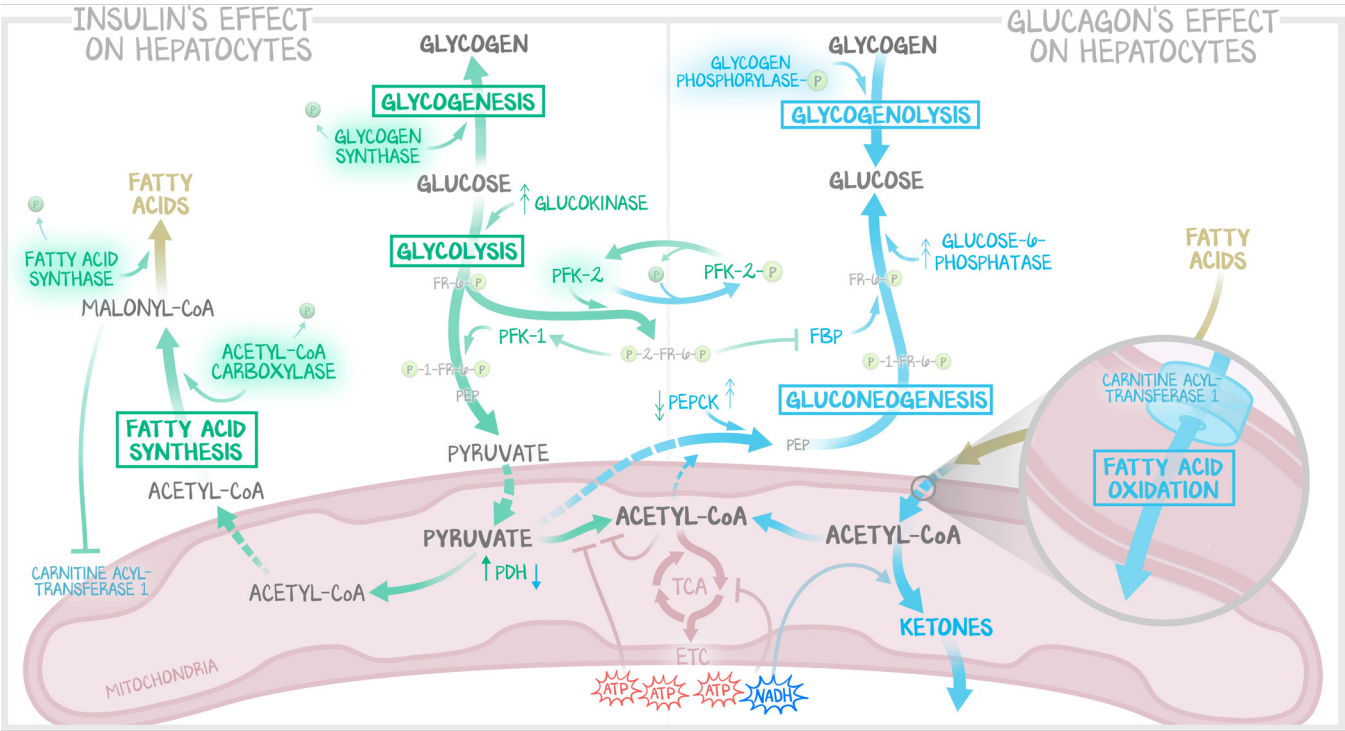

Figure 1.5: Insulin vs. Glucagon in Hepatocytes
Don't worry, this isn't biochemistry. Glycogen, glucose, and fatty acids are regulated by several key steps highlighted in the context of the limited biochemistry. Insulin stimulates glycogenesis by stimulating glycogen synthase. Fatty acid synthesis increases through increased expression of fatty acid synthase and activation of acetyl-CoA carboxylase. Acetyl-CoA carboxylase makes malonyl-CoA, which in turn inhibits fatty acid oxidation. Gluconeogenesis is prevented, and thus the default glycolysis stimulated, by stimulating PFK-2, which makes fructose-2,6-bisphosphate, which in turn inhibits fructose-1,6-bisphosphatase 1. Glucagon stimulates the breakdown of glycogen, the synthesis of glucose, and the consumption of fatty acids. Both the absence of insulin's signal and the presence of glucagon push hepatocytes this way. Glucagon induces the expression of glucose-6-phosphatase and PEPCK and phosphorylates and inactivates PFK-2, which favors fructose-1,6-bisphosphatase 1 activation, all favoring gluconeogenesis. Phosphorylation of glycogen phosphorylase stimulates glycogenolysis. Regarding fatty acids, glucagon has no direct effect—only the absence of insulin leads to fatty acid oxidation.

Fatty acids. Insulin stimulates the synthesis of fatty acids and the formation of triglycerides to be dispatched to and stored within adipocytes. Insulin upregulates the expression of **fatty acid synthase** and activates cytoplasmic **acetyl-CoA carboxylase** (the rate-limiting enzyme of fatty acid synthesis) through dephosphorylation. Fatty acids are synthesized from excess acetyl-CoA, packaged as triglycerides, and dispatched to the periphery as VLDL. Hormone-sensitive lipase cleaves the fatty acids from glycerol, allowing uptake of those fatty acids into the adipocyte, which assembles the triglycerides anew (using its own glycerol backbone from glycolysis).

Counter Regulatory Hormones

Growth hormone, epinephrine, and glucagon oppose insulin's actions. In the pancreas, glucagon and epinephrine stimulate β cells to release insulin. In **every other cell,** these hormones oppose the physiological action of the insulin receptor. Fatty acids are mobilized from adipocytes to the liver (**lipolysis**) to generate energy and ketones (**ketogenesis**). Amino acids are mobilized from skeletal muscle to the liver to be made into glucose (**protein catabolism**). In the liver, hepatocytes are induced to perform **gluconeogenesis** and glycogenolysis.

Glucagon

Glucagon is produced by α cells of the pancreatic islets. At physiological levels, glucagon mostly affects only the liver. In other tissues, it is the absence of insulin signaling that results in the metabolic effect of the fasting, glucagon-dominant state.

Glucagon stimulates the glucagon receptor. The glucagon receptor is a G protein-coupled receptor. Activation of the receptor leads to activation of the **G_s-AC-cAMP-PKA** pathway. Phosphokinase A (PKA), "kinases" things, aka phosphorylates things. Glucagon downregulates, phosphorylates, and inactivates the same enzymes that insulin downregulated, dephosphorylated, and activated—acetyl-CoA carboxylase, glycogen phosphorylase, glycogen synthase, PFK-2, fatty acid synthase.

In hepatocytes, fatty acid synthesis is inhibited (phosphorylation and inactivation of acetyl-CoA carboxylase and decreased expression of fatty acid synthase). That reduced activity of acetyl CoA carboxylase reduces the formation of malonyl-CoA. Malonyl CoA inhibits **carnitine acyl-transferase 1** (CAT1). The loss of insulin signal and the presence of glucagon both lead to the downregulation of acetyl-CoA carboxylase and, therefore, the downregulation of malonyl-CoA and subsequent upregulation of CAT1 (CAT1 becomes disinhibited as malonyl-CoA levels drop). Disinhibition of CAT1 increases fatty acid oxidation. A convoluted mechanism, but what it says is **glucagon = fatty acid oxidation**. Fatty acid oxidation provides the liver with ATP so it can perform gluconeogenesis. Once the liver is flush with high-energy molecules, excess fatty acids, and therefore excess acetyl-CoA, the liver is shunted towards **ketogenesis**.

In hepatocytes, **glycogenolysis is stimulated**. The phosphorylation and inactivation of glycogen synthase and the phosphorylation and activation of glycogen phosphorylase result in hepatic glycogen-o-lysis and glucose release.

In hepatocytes, **gluconeogenesis is stimulated**. Phosphorylation and inactivation of PFK-2 lead to the disinhibition of FBP (gluconeogenesis) and inhibition of PFK-1 (glycolysis). **PEPCK** and **glucose-6-phosphatase** gene transcription is increased.

Glucagon does increase **lipolysis** in adipocytes and **proteolysis** in skeletal muscle. However, these effects are only demonstrable with very high concentrations of glucagon. Thus, it is likely the loss of the insulin signal's induction of lipogenesis and protein anabolism that results in lipolysis and protein catabolism.

Pancreatic Hormone Excess

All excess hormone syndromes are caused by pancreatic masses autonomously secreting hormone. The one we spend the most time on is insulinoma. The others are more honorable mentions. If ever you actually see a patient with one of these tumors, screen for MEN1 syndrome.

Insulinoma. Although not a common diagnosis, insulinoma is a favorite of licensing examinations because it is a subject that crosses physiology, pharmacology, and pathology. In truth, the surreptitious use of exogenous insulin (inducing hypoglycemia) and sulfonylureas (inducing insulin expression

inducing hypoglycemia) is vastly more common than an autonomously acting insulin-secreting tumor. So much so that the Mystery Diagnosis case presented each year to the residents in Dr. Williams' residency program concluded with the admission that the case was altered to be an insulinoma and was based on a guy who was just taking his mother's diabetes meds and lying to the health care community about it. All residents are sad every time the truth comes out.

Excess insulin in the absence of consuming glucose will cause **hypoglycemia**. And that is the key feature of insulinoma—**symptomatic hypoglycemia** with **documented low blood glucose** and a **reversal of symptoms with IV dextrose**. When someone is suspected of having an insulinoma, they are brought into the hospital for a 72-hour fast. Hypoglycemia from insulinomas can be provoked by a fast, and rarely does hypoglycemia occur in response to a meal. So the person comes into the hospital, where they are observed. When they have hypoglycemia symptoms, labs are drawn, the capillary blood glucose is assessed, and IV dextrose is given. Symptoms can be altered mental status, seizure, palpitations, diaphoresis, whatever. The capillary glucose is performed to make sure their symptoms are from hypoglycemia and to get the dextrose ready. The venous lab draw will be used to confirm true hypoglycemia and screen for the tools of fabrication. **Insulin levels** and both a **sulfonylurea screen** and **C-peptide levels** are analyzed. The insulin is up, and the blood sugar low. If the C-peptide is also elevated, it means that the excess insulin came from within the person. If the C-peptide is low, the patient self-injected with insulin. If insulin comes from an insulinoma, it must also come with C-peptide. If the c-peptide is elevated, but the sulfonylurea screen is positive, then the patient ate a bunch of sulfonylurea (which induces insulin secretion). Only if the insulin is high, the blood sugar low, C-peptide is high, and sulfonylurea screen negative does the patient get a CT and resection of the insulinoma.

Glucagonoma. When the glucagon levels go up quite high, they induce mild diabetes mellitus and weight loss. That isn't generally going to cause any alarm, nor should you be checking glucagon levels in someone who has mild diabetes. The giveaway for glucagonoma is the characteristic skin rash called **necrolytic migratory erythema**. This diagnosis will take time to work itself out, as a skin rash does not draw attention very quickly. The rash tends to appear first on the lower extremities or groin, although it can also appear on the face. The rash moves (migratory) and is a blistering disease ("necrolytic erythema"). If biopsied, it will show necrosis. Most patients are treated for more common skin conditions and are given steroids without effect. The diagnosis is more often made by a dermatologist than an endocrinologist. High **plasma glucagon levels** are required for the diagnosis. CT of the pancreas identifies the lesion, and resection is usually curative.

Somatostatinoma. Somatostatin puts everything into "somatostasis." That means it turns off all the gut hormones. It is very difficult to intuit the symptoms, but we're going to try. Somatostatin turns off parietal cells in the stomach, leading to achlorhydria. This causes problems with the digestion of food and atrophy of the stomach on endoscopy. Somatostatin turns off CCK, thereby reducing gallbladder contractions. This causes bile not to be released into the duodenum, and subsequent fat malabsorption leading to steatorrhea. Because bile doesn't leave the gallbladder, but the gallbladder continues to reabsorb water from the bile, the bile becomes concentrated, leading to gallstones. It is a very rare diagnosis that causes a mishmash of mild GI symptoms. High **somatostatin levels** are required for diagnosis. CT of the pancreas rarely identifies the lesion. Instead, specialized **somatostatin receptor scintigraphy** is required to localize the tumor. Resection is curative.

Gastrinoma. Discussed in Gastrointestinal as the peptic ulcer-inducing Zollinger-Ellison syndrome. Gastrin is not normally secreted by the pancreas, so this condition is technically a paraneoplastic syndrome.